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May 17, 2001

BOX PCT

Assistant Commissioner
for Patents
Washington, D.C. 20231

PCT/ES00/00354-filed
September 21, 2000

Re: Application of **Eliseo QUINTANILLA ALMAGRO, Ana RAMIREZ BOSCA, August BERND, José PARDO ZAPATA, Joaquin DIAZ ALPERI, David PAMIES MIRA, Miguel Angel CARRION GUTIERREZ** and **Jose Miguel SEMPERE ORTELLS** entitled "**NEW PHARMACOLOGICAL ACTIVITIES OF *CURCUMA LONGA* EXTRACTS**"
ASAC COMPAÑÍA DE BIOTECNOLOGIA E INVESTIGACION, S.A.
Our Ref: **Q-64417**

Dear Sir:

The following documents and fees are submitted herewith in connection with the above application for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter I of the Patent Cooperation Treaty:

- ☐ an executed Declaration and Power of Attorney.
- ☑ the International Application (along with an English translation thereof).
- ☑ 8 sheets of drawings.
- ☐ an English translation of Article 19 claim amendments.
- ☐ an English translation of Article 34 amendments (annexes to the IPER).
- ☐ an executed Assignment and PTO-1595 form.
- ☐ a Form PTO-1449 listing the ISR references, and a complete copy of each reference.
- ☑ a Preliminary Amendment.

Priority is claimed from **September 23, 1999**, based on Spanish Application No. **P 9902364**.

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The Declaration and Power of Attorney, Assignment, Small Entity Status Declaration, and Information Disclosure Statement (along with Form PTO-1449 listing the International Search Report (ISR) references and a complete copy of each reference) will be submitted at a later date.

It is assumed that a copy of the International Application, the English translation of the International Application and priority document, as required by § 371(c), will be supplied directly by the International Bureau. However, for the Examiner's convenience a copy of International Application, and English translation thereof, is provided herewith.

Applicants claim benefit of small entity status in accordance with 37 C.F.R. § 1.27.

The Government filing fee is calculated as follows:

Total claims	10	- 20 =		x	$9.00	=	$.00
Independent claims	8	- 3 =	5	x	$40.00	=	$200.00
Base Fee							$500.00
TOTAL FEE							$700.00

A check for the statutory filing fee of $700.00 is attached hereto.

However, the Assistant Commissioner is hereby directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880.

The Assistant Commissioner is also hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.492 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Respectfully submitted,

Gordon Kit
Registration No. 30,764

2688 SY/US

Applicant or Patentee: Eliseo QUINTANILLA ALMAGRO
Application No. 09/856,035
Filed or Issued: May 17, 2001
For: New Pharmacological Activities of Curcuma Longa extracts

Attorney's Docket No. Q-64417

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27 (c)) – SMALL BUSINESS CONCERN

I hereby declare that I am

☐ the owner of the small business concern identified below:
☑ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN ASAC, COMPAÑIA DE BIOTECNOLOGIA E INVESTIGACION, S.A.
ADDRESS OF CONCERN 14, Calle Sagitario, 03006 ALICANTE, Spain

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CRF 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41 (a) and (b) of Tittle 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third or parties controls or has the power to control both.

I hereby declare that rights under contact or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled: by inventor .

Described in

☐ the specification filed herewith
☑ application no. 09/856,035 filed May 17, 2001
☐ patent no. _____ issued _____

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statement are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME _____
ADDRESS _____

☐ INDIVIDUAL ☑ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any charge in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Eliseo Quintanilla Almagro

TITLE IN ORGANIZATION: General Director

ADDRESS OF PERSON SIGNING: As above 14, Calle Sagitario, 03006 Alicante, Spain

Signature: [signature] Date: February 14, 2002

A.S.A.C. CIA. DE BIOTECNOLOGIA E INVESTIGACION, S.A.
C/. Sagitario, 03006 Alicante

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Eliseo QUINTANILLA ALMAGRO et al

	CHAPTER I of		
Appln. No.:	**PCT/ES00/00354**	Group Art Unit:	**0000**
Filed:	**May 17, 2001**	Examiner:	**Unknown**

For: **NEW PHARMACOLOGICAL ACTIVITIES OF *CURCUMA LONGA* EXTRACTS**

PRELIMINARY AMENDMENT

Assistant Commissioner
of Patents
Washington, D.C. 20231

Sir:

Prior to examining the above-identified application, please amend the application as follows.

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 1, between lines 2 and 3, insert

-- This application is a § 371 of PCT/ES00/00354, filed September 21, 2000. --

IN THE CLAIMS:

Please cancel Claims 1-10.

Please add the following new claims:

-- Claim 11. A method of photosentivization comprising administering, to a subject in need thereof, a

photosensitivization effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 12. The method of Claim 11, wherein said administering occurs after radiating said subject with visible light.

Claim 13. A method of photosensitivization comprising administering, to a subject in need thereof, a photosensitivization effective amount of an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 14. The method of Claim 13, wherein said administering occurs after radiating said subject with visible light.

Claim 15. A method of inhibiting proliferation of cells comprising contacting said cells with an antiproliferative effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 16. A method of inhibiting proliferation of cells comprising contacting said cells with an antiproliferative

effective amount of an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 17. A method for inhibiting cellular secretion of cytokines IL-6 and IL-8 comprising irradiating cells with UV light so as to induce secretion of IL-6 and IL-8, and thereafter contacting the resulting irradiated cells with a cytokine inhibiting effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 18. A method for the treatment of psoriasis comprising administering, to a subject afflicted with psoriasis, a therapeutically effective amount of an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 19. A method for inhibiting plasma fibrinogen comprising administering, to a subject in need thereof, an effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 20. A method for reducing Apolipoprotein B/ApolipoproteinA-1 quotient comprising

administering, to a subject in need thereof, an effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction. --

REMARKS

The specification has been amended to insert formal matter, Claims 1-10 have been deleted and new Claims 11-20 have been substituted therefor (in order to make the claims consistent with U.S. patent practice). Hence, the amendment to the specification, cancellation of Claims 1-10 and substitution of new Claims 11-20 do not constitute new matter.

In view of the above, allowance is respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

Gordon Kit
Registration No. 30,764

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Date: May 17, 2001

A P P E N D I X

Marked-up Version of Changes

IN THE SPECIFICATION:

Page 1, between lines 2 and 3, the following is inserted.

-- This application is a § 371 of PCT/ES00/00354, filed September 21, 2000. --.

IN THE CLAIMS:

Claims 1-10 are being cancelled.

New Claims 11-20 is added:

-- Claim 11. A method of photosentivization comprising administering, to a subject in need thereof, a photosensitivization effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 12. The method of Claim 11, wherein said administering occurs after radiating said subject with visible light.

Claim 13. A method of photosensitivization comprising administering, to a subject in need thereof, a photosensitivization effective amount of an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

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Claim 18. A method for the treatment of psoriasis comprising administering, to a subject afflicted with psoriasis, a therapeutically effective amount of an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 19. A method for inhibiting plasma fibrinogen comprising administering, to a subject in need thereof, an effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction.

Claim 20. A method for reducing Apolipoprotein B/ApolipoproteinA-1 quotient comprising administering, to a subject in need thereof, an effective amount of a hydroalcoholic extract of *Curcuma longa* consisting of:

(A) an alcoholic extract of *Curcuma longa*, wherein said alcoholic extract comprises curcuminoids; and

(B) an aqueous extract of *Curcuma longa*, wherein said aqueous extract comprises a protein fraction. --

NEW PHARMACOLOGICAL ACTIVITIES OF *CURCUMA LONGA* EXTRACTS

TECHNICAL FIELD OF THE INVENTION

This invention describes the new pharmacological activities of *Curcuma longa* extracts as an antiproliferative agent with an effect similar to betamethasone-17-valerate and a photosensitivisation effect greater than psoralens and its use in a pharmaceutical composition as an agent in proliferative diseases such as psoriasis, mycosis fungoides, atopic dermatitis, and photodermatosis without the side effects caused by psoralens, corticoids and/or retinoids. The photosensitivisation effect is active both with ultraviolet light and visible light.

This invention refers to a new pharmacological effect of Curcuma extracts as an antiatheromatous agent, reducing plasmatic fibrinogen and the apolipoprotein B/apolipoprotein A-I quotient, without altering the other coagulation parameters.

STATE OF THE ART

Psoriasis is a chronic inflammatory dermatitis of unknown aetiology. Clinically, it is characterised by papulous lesions on erythemato-scaly maculae. The majority of these lesions are due to alterations in cellular proliferation marked by immunological and genetic mechanisms.

We find an increase in arachinonic acid and its derivatives, both in normal and diseased skin; an increase in polyamines, an increase of B4 leukotriene in the scales. From the epidermis and the dermis we find an increase in Langerhans cells with lower infiltration of CD8 lymphocytes compared with CD4. These patients' neutrophils synthesise double the number of B4 leukotrienes as healthy individuals.

IL-6 interleukin is a cytokine that structures the 2(BSF-2) factor, structurally identical to interferon ß-2 (IFN- ß-2). IL-6 is synthesised in the fibroblasts, monocytes and T cells. This cytokine stimulates the acute phase of protein synthesis and the production of immunoglobulins.

IL-8 is an interleukin that is directly involved in psoriasis, since it is responsible for producing the migration of the neutrophils that are produced in the epidermis and consequently increases the inflammatory process.

In the present therapy used for psoriasis it is fundamental to act on cellular proliferation and the production of cytokines by the use of glucocorticoids and/or photosensitivisation agents (psoralens).

Cell cultures are acknowledged models for the study of cell physiology and the effect of drugs. HaCat cells are derived from human keratinocytes that exhibit the same differentiations as normal keratinocytes. Therefore, HaCat cells are an extraordinary model for testing different substances for topical application.

Keratinocytes are very biologically active cells, the function of which is not only to produce keratin synthesis to form the corneal stratus, but which also have immunological properties based on the production and secretion of cytokines and the selective expression of surface receivers.

Different stimulants including ultraviolet radiation have inflammatory responses that act directly on these keratinocytes, producing a release of cytokines and adhesion molecules. This production of substances on the epidermis level starts the cutaneous inflammation symptoms, releasing the IL-6 and the IL-8, which are two cytokines involved in inflammatory cutaneous processes.

Glucocorticoids are the substances most used in the dermatology field, because of their immunosuppressant and anti-inflammatory properties, manifest after UV radiation, but with no effect in visible light.

Different studies show that corticoids affect the production of pro-inflammatory cytokines. Well known glucocorticoids such as hydrocortisone-17-butyrate and betametasone-17-valerate produce a decrease in inflammatory cytokines after ultraviolet radiation.

The accessibility of the skin often allows for skin alterations to be treated by the topical application of drugs. Topical corticoids, thanks to their anti-inflammatory, vaso constricting and antimycotic properties, have been seen to be useful in a large variety of dermatosis. Nevertheless, the application of corticoids has a series of side effects that have a direct impact on the skin:

- Cutaneous atrophies, which consist of thin, transparent skin, purple lesions, star-shaped scars and elastic catabolic striae.

- Delay in scar formation because of inhibition of the fibroblasts' function.

- Disguise and de-typing of cutaneous infections, particularly dermatophytosis, making diagnosis difficult and with the possible appearance of viral or bacterial cutaneous infections.

- Skin pigmentation disorders with hyper or hypopigmentation.

- Contact dermatitis.

- Habituation and tachyphylaxis phenomena that require the use of increasingly strong products and lead to relapses with the appearance of increasingly severe forms of the process (pustular psoriasis) that could be caused by suddenly ceasing administration.

Systemic side effects are fortunately less frequent, since the use of corticoids for long periods is required, as for psoriasis. The most common side effects are:

- Inhibition of the hypothalamus - hypophysial - suprarenal axis.
- Episodes of hyperglucaemia and glucaemia.
- A fall in the number of eosinophils.
- Clinical manifestations of Cushing's Syndrome.

Other therapies used for psoriasis are the oral or topical application of photosensitivisation substances (psoralens) together with ultraviolet A radiation. The photochemistry of psoralens is not well know, and can act on several levels. Psoralens bind with DNA and RNA, but interact with lysosomes, endotheliums, cytoplasmatic membranes and dermic cells. In the dark, psoralen is intercalated between the DNA bases. With UVA, cyclobutane monoadducts are produced by binding with a DNA base thymine or cytokine. If radiation continues, a new photon stimulates the other double psoralen link to form a crossover link with the thymine from the other DNA chain. The formation of these bifunctional adducts suppresses DNA synthesis. Another reaction that is observed is that the photoactivated psoralen can act with molecular oxygen to produce an oxygen singlet, superoxide anion and free radicals, and all these reactive forms act on the keratinocytes. The use of psoralens, therefore, presents side effects that are well known in dermatological literature, such as a decrease in delayed immunity, phototoxic reactions, immunosuppression, a decrease in the production of IL-1 by the keratinocytes and more inclination to skin cancers.

On the other hand, photosensitivisation substances can be used in the treatment of different diseases with

an excess of hyperproliferation such as vitiligo, atopic dermatitis, granuloma annulare, lichen, mycosis fungoides, lymphomas, leukaemia, etc.

One of the greatest coronary risk factors is the plasmatic concentration of fibrinogen. Stone and Thorp *J. Royal College Gen Practitioners* **35**,565-569 (1985), showed that in men between 40-60 years of age there is a relation between heart attacks and the plasmatic levels of fibrinogen. Particularly in men with high cholesterol and high arterial pressure, heart attack frequency was 6 and 12 times greater, respectively, in individuals with high levels of fibrinogen compared with individuals with low fibrinogen levels. In multivariable models, then, fibrinogen concentration is at least as important as other risk factors for cardiac diseases, such as cholesterol, smoking and arterial pressure. Another demonstration of the pathogenetic role of fibrinogen and its products has been described by Kaplan and Bini. *Arteriosclerosis* **9**, 109 (1989). They conclude that fibrinogen is involved in atheroma plaques. The study of atheroma plaques by anti-body fluorescence (with antifibrinogen polyclonal anti-bodies) shows fibrinogen or fibrin in a wide range of atherosclerotic lesions.

Sadoshima and Tanaka. *Atherosclerosis* **34**, 93-97 (1979) have also shown an accumulation of fibrinogen and LDL in human cerebral arteries. In early atheroma plaques fibrinogen is observed in the interstices of the intima and between the duplicated internal elastic laminae. The authors describe the accumulation of fibrinogen in the intima before the LDL and therefore fibrinogen is a greater risk factor than LDL.

Consequently, a drug capable of reducing the plasmatic concentration of fibrinogen would be useful

for the treatment and/or prophylaxis of cardiovascular diseases.

The drugs used as fibrinogen reducers (salicylic acid, cumarin derivatives) increase fibrinolytic activity, and have side effects on coagulation parameters (Quick's index, thrombin time, prothrombin time, ATPP).

Apolipoprotein B is the fundamental protein component of low density lipoproteins (LDL), so that high concentrations of this protein indicate a large amount of LDL. It is known that this lipoprotein, when it oxidises, is captured by the macrophages and its transformation into foam cells is determinant for the start of atheroma plaques. The more apolipoprotein B there is in plasma, then, the greater the risk of atheroma.

Apolipoprotein A-I is a fundamental particle component in high density lipoproteins (HDL). Its function is to activate the lecithin-cholesterol-acyl-transferase enzyme, in charge of forming cholesterol esters from the free cholesterol from the peripheral tissues and the phospholipids that form the HDL particle itself. These lipoproteins are fundamental for the maintenance of cholesterol homeostasis, since the remove the cholesterol accumulated in the peripheral tissues and they take it to the liver, where it is eliminated as biliary salts or recirculated to other lipoproteins. High concentrations of Apo A-I, therefore, indicate less risk of atheroma.

The Apo B/Apo A-I ratio is therefore a better indicator of risk of atheroma, since the direction of the changes in atherosclerosis processes usually give rise to an increase of Apo B and a decrease of Apo A-I.

Curcumin and the curcuminoids present in the rhizomes of *Curcuma longa* and the Zingiberaceae family

in general, have been used for the treatment of a large variety of diseases. Examples are US 5891924 (inhibitor of NF kappa B activation), US 5336496 (inhibitor of delta 5 desaturase), EP 256353 (treatment of bad absorption syndromes), EP 568001 (anti-viral agent), US 5108750 (hyperlipidaemia and platelet aggregation reducer), FR 2655054 (cell protector) and EP 550807 (antioxidant and anti-inflammatory properties), EP440885 (anti-inflammatory), EP 319058 (against hair loss), US 510750, US 4906471 and US 4842859 (anti-platelet aggregation and anti-cholesterol agent), WO 88/05304 (treatment of neurological disorders), W 96/03999 (lipidic peroxide reducer), ES 20103689 (modulates high and low density oxidised lipoproteins, protects keratinocytes against free radicals and increases cell proliferation in aged human tissue). Chinese patent CN1156601 describes the use of medicinal composition prepared from 13 plants, including *Curcuma longa*, as a reducing agent for triglycerides and cholesterol increasing the HDL.

There is a large number of documents in the scientific literature, describing different pharmacological activities such as anti-tumour agent, anti-inflammatory, scar forming, proliferation inhibitor, anti-fungal, etc.

The aqueous extract of *Curcuma longa*, free from curcuminoids, has also been seen to have antioxidant properties. Srinivas et al. *Archives of Biochemistry and Biophysis* **292** n°2 617-623 (1992), describe the antioxidant activity of turmerin, a protein that is present in Curcuma rhizomes. Yeharayou et al *Ind J. Med. Res.* **64**, 4, 601 (1976), describe the anti-inflammatory effect of the aqueous extract of *Curcuma longa*, with properties similar to hydrocortisone. Gonda et al. *Chem Pharm Bull* **40**, 990 (1992) describe the

immunological activity of ukonan A and its degradation products.

The document that is closes to our invention, Tonnessen et al *J. Pharm Sci* **76**, n°5 (1987), describes the phototoxic activity of curcumin in coreless biological systems (E. Coli, Salmonella typhimuis); however, this document comments on the possible mutagenic effects on DNA.

Dhal et al *Photochemistry and Photobiology* **59** n° 3, 290 (1994) describe the phototoxic activity of curcumin on rat cells.

None of the documents of the state of the art describes the photosensitivisation properties of aqueous *Curcuma longa* extracts, the inhibition of the secretion of inflammatory cytokines after UVA and/or visible radiation with aqueous *Curcuma longa* extracts and/or the beneficial effects on a clinical and histological level of a pharmaceutical composition the active ingredient of which is the aqueous *Curcuma longa* extract for different types of psoriasis with oral and topical application.

The development of this new pharmacological activity of *Curcuma longa* extract means that it is an ideal drug for the treatment of diseased with cell hyperproliferation such as psoriasis, without the side effects of the presently used treatments (psoralens, corticoids). Moreover, the photosensitivisation properties of Curcuma extracts with visible lights avoids the possible mutagenic effects of ultraviolet A or B radiation.

Neither does the state of the art describe the activity of *Curcuma longa* extracts as reducers of fibrinogen and the Apolipoprotein B /Apolipoprotein A-I quotient. The development of this new pharmacological activity of *Curcuma longa* extract means that it is an

ideal drug for the treatment of atherosclerosis and cardiovascular diseases, without altering coagulation parameters.

The documents that are closest to our invention describe the lipidic peroxide reducing effect and the cholesterol reducing effect. These are etiopathogenic factors of the atherosclerotic process. However, these factors are unrelated.

Vitamin C and vitamin E, drugs that gave anti-oxidant and lipidic peroxide reducing properties, have had no effect on the plasmatic concentration of fibrinogen in humans after their administration. (Bates et al *J Hypertens*.Jul; **16** (7): 925-32 (1998)). Moghadasian et al *Circulation* Apr 6; 99 (13): 1733-1739 (1999) conclude that the probucol, which is known to have hypolipemiant and antioxidant capacity, increases the plasmatic concentrations of fibrinogen, showing proatherogenic activity. Rifici et *al Thromb Haemost* Sep; **78** (3): 1111-4 (1997) show that the lipooxidation produced by antioxidant vitamins does not alter the fibrinolitic activity.

The use of the vegetable extracts of plants with pharmacological activities is well known, and it is also known that the active ingredients can be isolated and purified from plant extracts. However, active ingredients that are purified and/or synthetically obtained could have side effects or be toxic, such as in the case of atropine, digitalis, nicotine etc.

Vegetable extracts contain a series of structurally related chemical species due to the metabolic processes in plants. These related compounds can have a synergic effect on pharmacological activity. These chemical substances are used as markers, in order to qualitatively and quantitatively standardise the extracts. The alcoholic extracts of Curcuma are

chemically characterised in that they contain curcuminoids (curcumin, desmetoxicurcumin and bisdesmetxosicurcumin). The aqueous extract of Curcuma is characterised in that it does not contain curcuminoids, but a protein fraction and a polysaccharide fraction, in which ukonan A, B and C have been identified. The pharmacological effect is due to the total composition of the aqueous and/or alcoholic extract of *Curcuma longa*.

PURPOSE OF THE INVENTION

This invention develops a new therapeutic application of the aqueous extract of *Curcuma longa* as a photosensitivisation agent, an antiproliferative agent and for use in diseases with an excess of cell proliferation, both with ultraviolet and visible light.

This invention develops a new therapeutic application of *Curcuma longa* extracts as a reducer of the plasmatic levels of fibrinogen, lowering them to normal values in healthy individuals and reducing the apolipoprotein B /apolipoprotein A quotient.

DETAILED DESCRIPTION OF THE INVENTION

The alcoholic extract of *Curcuma longa* can be obtained, according to Spanish patent ES 2103689, by the extraction of the Curcuma rhizomes by macerating with alcohol (methanol, ethanol) at 50°C for 24 hours and then removing the solvent at reduced pressure. The alcoholic extract of *Curcuma longa* is chemically characterised in that it contains curcuminoids. Alternatively, other extraction and/or purification methods known by an expert can be used, such as extraction with other organic solvents, extraction with solvents in a supercritical state, reflux extraction and steam current extraction. The extract can be

purified by fractioned crystallisation, chromatography, liquid-liquid extraction, etc.

The aqueous extract of Curcuma can also be obtained by macerating with water for 24 hours at 50 - 70°C and then removing the solvent at reduced pressure. The aqueous extract of *Curcuma longa* is chemically characterised in that it contains a protein fraction with a concentration around 20-30%, measured by the Pierce method, analysing the protein nitrogen, and a polysaccharide content (ukonan A, B and C) between 3-8%, with no curcuminoids. The procedure for manufacturing *Curcuma longa* extracts is not the object of this invention.

Alternatively, combinations of the two extracts can be used, obtaining hydroalcoholic extracts chemically characterised by the concentration of their markers (concentration of curcuminoids, proteins and polysaccharides).

The content of the markers can be measured by the methods described in the state of the art. The curcuminoids can be quantified by visible-ultraviolet spectrophotometry at 420 nm, the protein fraction can be quantified by the Pierce method, analysing the protein nitrogen and/or by liquid chromatography and the polysaccharide fraction is quantified by liquid chromatography.

The hydroalcoholic extract of *Curcuma longa* has shown a pharmacological activity greater than curcumin (greater proliferative activity, greater photosensitivisation activity, greater inhibition of cytokine secretion). These results support the view that vegetable extracts are drugs that are different than the molecules responsible for pharmacological activity, because the pharmacodynamics are different (absorption, distribution, action and elimination), and

there could be synergic or anti-synergic effects between the different chemical species present in the extract. The hydroalcoholic extract of *Curcuma longa* has shown an antiproliferative activity similar to betametasone-17-valerate. This hydroalcoholic extract showed a significant decrease in the incorporation of 5-bromine-2´-deoxyuridin (BrdU) in the DNA of human keratinocyte cultures between concentrations of 5 µg/ml and 50 µg/ml of extract. This effect is similar to that of betametasone-17-valerate.

Both the aqueous extract of Curcuma and the hydroalcoholic extract of *Curcuma longa* have inhibited the secretion of cytokine IL-6 and/or IL-8 in human keratinocyte cultures with an activity similar to betametasone-17-valerate. This inhibition is increased after subjecting the cells to ultraviolet A radiation.

The aqueous and hydroalcoholic extracts of Curcuma have been seen to inhibit cell proliferation without altering the mitochondrial activity, and the extracts have no effect on protein synthesis. The extract therefore shows cytostatic activity.

On the other hand, the hydroalcoholic extracts show photosensitivisation activity and can therefore be used in proliferative diseases such as psoriasis, vitiligo, lymphomas, mycosis fungoides, etc, instead of psoralens.

In studies carried out on eucaryote cells (human ketinocytes) with *Curcuma longa* extracts, the activated curcumin has been found in the cytoplasm. Therefore the nucleus is free from curcumin and the extract does not interact with the nuclear DNA and the secondary and mutagenic effects produced by psoralens do not appear.

The hydroalcoholic extract (10% curcuminoids, 18% protein fraction, 3% polysaccharides) of Curcuma shows

a greater photosensitivisation activity after UVA radiation than curcumin.

Therefore, a smaller amount of the drug is best for a greater photosensitivisation activity (lower percentage of BrdU incorporated).

% incorporation	80	60	40	20
Ng extract	2000	4000	5000	6000
Ng curcumin equiv	200	400	500	600
Ng curcumin	600	800	1000	1200

To produce the same level of photosensitivisation as Curcuma extracts, doses of 10 ng/ml of psoralen is required, as with this dose toxic and mutagenic effects are produced.

The administration of a cream the active ingredient of which is the aqueous extract of *Curcuma longa* at 2%, and one tablet a day with 100 mg of aqueous extract with pharmaceutically acceptable excipients has been seen to be clinically effective in different types of psoriasis, and these effects are increased after radiation with ultraviolet A light. There are no side effects, as is the case for corticoids.

22 patients with different types of psoriasis were studied: Guttate, Vulgar, Inverse, Palmo-plantar, Pustular. They were without any psoriasis treatment (retinoids, corticoids, etc.) for 15 days. The cream with aqueous Curcuma extract was then applied and a tablet was administered every 12 days. The cream was tolerated perfectly by all the patients and no patient had to cease treatment because of cutaneous or systemic adverse reactions.

All the patients and all the types of psoriasis responded to this therapy. In the palmo-plantar psoriasis, which does not react to conventional

treatments, all the patients responded to the treatment. In the vulgar psoriasis, the plaque was reduced after administration. Fissured and/or ulcerated pustular psoriasis scarred quickly. An antiseptic and drying effect was observed in the inverse psoriasis.

The association of the aqueous Curcuma extract with UVA favoured the product's activity, whitening the lesions after three days of treatment.

The hydroalcoholic (10% curcuminoids, 18% protein fraction, 3% polysaccharides) extract of Curcuma has shown photosensitivisation activity with visible light, inhibiting the percentage of BrdU incorporated into the DNA after radiation with visible light in human keratinocyte cultures.

The administration of a cream in which the active ingredient is the hydroalcoholic extract of *Curcuma longa* at 2% with pharmaceutically acceptable excipients has been shown to be clinically effective in the different types of psoriasis that did not respond to treatment with corticoids or with PUVA. After 15 days of treatment with the cream with hydroalcoholic extract of *Curcuma longa*, the erythema, the infiltration and the scaling disappeared. The effects were greater after radiation with visible light and there were no side effects, unlike with the use of psoralens and ultraviolet light.

Curcuma longa extracts (50 mg of alcoholic extract and 50 mg of polar extract of *Curcuma longa*), equivalent to 10 mg of curcuminoids and 15 mg of proteins and 2 mg of polysaccharides) together with pharmaceutically acceptable excipients, administered for 30 days, 2 tablets a day to 30 healthy individuals (16 men-14 women) aged between 24 and 75, showed a significant reduction in the fibrinogen levels. The values at the end of treatment were between 240-290

mg/dl. After administering the Curcuma extracts, the levels of fibrinogen in plasma fell to standard levels. In other words, Curcuma extracts have no fibrinolitic activity, merely reducing the levels of fibrinogen in individuals with high fibrinogen levels.

Values of 809, 690, 584, 490 mg/dl of fibrinogen became values of 241, 240, 290, 272 mg/dl after the treatment.

Coagulation parameters such as Quick's index, thrombin time, ATPP and prothrombin time did not experience significant changes and the values at the end of treatment were within reference values.

No side effects such as haemorrhages, nausea, vomiting, etc., were not observed. No tablets were observed to be toxic.

The following examples illustrate the invention, but they are not exhaustive to the scope of the invention.

BRIEF EXPLANATION OF THE FIGURES

Figure 1- Inhibition of the secretion of IL-6 and IL-8 after ultraviolet light radiation of aqueous Curcuma extract (ZCL3) and betametasone-17-valerate (B-17-V).

Figure 2- Inhibition of the secretion of IL-6 and IL-8 after ultraviolet light radiation of hydroalcoholic Curcuma extract (ZCL4) and betametasone-17-valerate (B-17-V).

Figure 3- Incorporation of BrdU of hydroalcoholic Curcuma extract (ZCL4) and betametasone-17-valerate (B-17-V).

Figure 4- Effect of aqueous Curcuma extract (ZCL3) on the incorporation of BrdU in the DNA after UV radiation. Photosensitive capacity.

Figure 5- Effect of hydroalcoholic Curcuma extract (ZCL4) on the incorporation of BrdU in the DNA after UV radiation. Photosensitive capacity.

Figure 6- Effect of curcumin on the incorporation of BrdU in the DNA after UV radiation. Photosensitive capacity.

Figure 7- Effect of psoralen on the incorporation of BrdU in the DNA after UV radiation. Photosensitive capacity.

Figure 8- Effect of the hydroalcoholic extract of *Curcuma longa* on the incorporation of BrdU in the DNA of human keratinocytes with visible light radiation (450 nm) and without radiation, with the concentration in µg/ml of extract represented on abscissas and the percentage of BrdU incorporation on ordinates. Photosensitivisation capacity with visible light.

EXAMPLES

Example 1. Variation of plasmatic fibrinogen and coagulation parameters after Curcuma intake.

The effect of Curcuma extracts on fibrinogen and coagulation parameters was studied in a total of 30 healthy individuals (16 men and 14 women) between 24 and 75 years of age with a good state of health.

At time zero blood was extracted from the ulnar vein and plasmatic fibrinogen was determined by the Clauss coagulation method (Clauss A. *Acta haemat* 1957;17: 237), together with the coagulation parameters.

2 tablets a day were administered for 15 days. After 30 days of treatment the plasmatic fibrinogen and the coagulation parameters were measured once again.

-Composition of each tablet:

Hydroalcoholic *Curcuma* extract	100.0 mg *
Microcrystalline cellulose	490.8 mg
Corn starch	45,0 mg

Aerosil	1.5 mg
Primojel	22.5 mg
Encompress	15.0 mg
Magnesium stearate	10.1 mg

*Equivalent to no less than 10 mg of curcuminoids, 15 mg of protein fraction and 2 mg of polysaccharides.

Following are the results obtained:

SEX	FIBRINOGEN T=0	FIBRINOGEN T=30
M	228	215
F	263	267
M	237	215
F	245	272
F	173	250
M	256	216
M	354	335
F	220	243
M	216	210
M	205	221
M	226	371
M	189	168
F	251	282
F	216	216
F	251	302
M	191	191
M	476	272
F	302	218
F	243	187
M	207	201
M	232	305
M	296	296
M	809	241
F	237	409
M	254	267
F	480	268

F	690	240
M	584	290
F	490	272
F	490	272

Fibrinogen reference values: 150 - 450 mg/dl.

The Curcuma extract normalised pathological fibrinogen values to reference values.

Variation of coagulation parameters after the intake of *Curcuma longa*

Prothrombin time		Quick's I		APTT		Thrombin time	
T=0	T=30	T=0	T=30	T=0	T=30	T=0	T=30
13.3	13.3	85.9	85.9	29.3	30.6	16.6	17.0
12.3	13.5	95.7	84.2	30.9	28.1	15.9	11.0
13.2	13.4	86.8	85.1	29.3	31.2	17.3	15.9
14.2	14.1	78.7	74.0	37.9	34.4	16.6	16.8
13.8	13.6	81.7	83.4	30.7	26.5	16.6	12.0
13.1	14.2	87.7	78.7	31.2	33.5	16.4	16.2
13.2	12.8	86.8	90.6	30.8	31.3	17.0	16.2
13.3	12.8	85.9	90.6	30.1	31.4	15.9	15.9
13.5	14.1	84.2	79.4	30.4	30.3	16.9	16.3
14.8	13.8	74.5	81.7	30.6	32.3	16.3	16.0
12.9	13.7	86.6	82.5	33.9	30.0	15.9	15.8
14.6	13.9	75.8	80.9	34.7	31.8	17.2	16.3
13.5	12.3	84.2	95.7	36.8	30.2	17.1	16.4
13.7	13.7	82.5	82.5	29.5	29.5	16.7	16.7
13.0	13.5	88.7	84.2	31.2	29.8	17.3	16.7
14.4	14.4	77.2	77.2	33.3	33.3	17.1	17.1
12.0	14.9	99.1	73.8	31.3	29.3	15.9	15.5
14.0	13.9	80.2	80.9	29.9	33.2	16.8	15.2
12.9	14.3	89.6	77.9	30.3	32.3	15.9	16.3
14.3	13.9	77.9	80.9	35.2	32.8	17.3	17.3
13.8	12.2	81.7	96.9	30.6	28.9	16.2	16.7
12.6	12.6	92.6	92.6	33.0	33.0	15.9	15.9

13.7	13.5	82.5	84.2	29.1	29.1	16.1	17.0
13.1	11.6	87.7	100	30.2	30.5	16.2	14.0
13.0	12.4	88.7	94.7	28.7	28.9	16.2	16.2
14.8	13.8	89.6	90.9	32.9	29.9	16.1	15.9
15.2	14.0	83.2	89.8	31.6	30.1	17.2	14.9
13.9	13.9	86.8	100	34.2	32.4	15.4	16.0
14.6	12.8	70.9	88.8	30.8	28.8	15.9	16.3
13.8	14.1	84.8	90.4	33.7	30.0	16.1	14.8

Reference values:

Prothrombin time: 10 - 20 seconds

Thrombin time: 10 - 20 seconds

Quick's Index: 75 - 100

APTT: 28-40 seconds.

After treatment, all the values are within the reference values.

Example 2 .Variation of the apolipoprotein B/apolipoprotein A quotient after the intake of Curcuma tablets.

The effect of Curcuma extracts on apolipoproteins A-1 and B was studied in a total of 13 healthy individuals of between 24 and 75 years of age, with a good state of health.

At time zero blood was extracted from the ulnar vein and apolipoproteins A-I and B were determined nephelometrically and their quotient was then calculated. After 15 days of treatment, as in example 1, apolipoproteins A-I and B were analysed once again.

Apo B		Apo A		ApoB/ApoA	
T=0	T=30	T=0	T=30	T=0	T=30
100	120	104	100	1.04	0.83
120	160	141	110	1.17	0.68
157	172	136	126	0.86	0.73
130	141	96	90	0.73	0.63
146	160	65	60	0.44	0.38

155	166	70	53	0.46	0.32
90	138	101	82	1.10	0.59
125	164	99	74	0.79	0.45
110	151	86	76	0.78	0.50
160	179	109	88	0.68	0.49
99	133	102	90	1.03	0.67
104	141	116	103	1.10	0.73

After the intake of *Curcuma longa* extract we observe a significant decreasing trend for the ApoB/ApoA quotient.

Example 3 Effect of aqueous Curcuma extract on psoriasis

Quantitative composition of the cream:

Aqueous Curcuma extract *	2 %
Greasy phase	27 %
Emulgents	47 %
Humectants	20 %
Preservatives	1%
pH adjusters	1%
Water	sq

*Content in proteins no less than 15%, content in polysaccharides no less than 4%.

22 patients diagnosed with psoriasis were studied, distributed by age and sex.

Sex	Age	Type of psoriasis
F	12	Guttate
F	22	Vulgar
F	37	Palmo-plantar
M	24	Vulgar
M	48	Vulgar
F	51	Inverse
F	27	Palmo-plantar
M	19	Vulgar

M	57	Palmo-plantar
M	61	Inverse
F	46	Palmo-plantar
M	6	Pustular
M	16	Vulgar
F	32	Vulgar
F	39	Pustular
F	41	Vulgar
M	31	Palmo-plantar
F	13	Guttate
F	3	Vulgar
F	51	Vulgar
F	60	Inverse
F	19	Palmo plantar

Criteria for inclusion:

- Patients clinically or histologically diagnosed with psoriasis.
- They had no other disease.
- They did not receive treatment for psoriasis.

Protocol:

The 22 patients went for 15 days without treatment of any kind, emollients, corticoids, retinoids, fatty acids.

Patients were instructed to apply the formula 3 times a day with a light massage and take 1 tablet every 12 hours.

Results:

All the patients tolerated the treatment well. The cream presented no irritation or contact reaction.

The cases of guttate psoriasis evolved in the same way. Their lesions were not very scaly but very erythematous. After 7 days of treatment there were no scaled and the erythema was minimal. After 14 days the

lesions were not visible. There were no residual pigmentation lesions.

4 of the 6 cases of psoriasis palmo-plantar had the palms more evidently affected, with scaly lesions and significant fissuration. After 7 days of treatment the fissuration, painful for the patients, had disappeared and been replaced by an erythematous lesion with badly defined borders with practically no scales. After 14 days, the lesions had been reduced to a slightly erythematous macula on skin with normal characteristics. The plantar lesions presented an important hyperkeratosis with fissuration and were more resistant to treatment, obtaining results after 14 days, with scarred fissures.

In the two patients with pustular psoriasis, the lesions scarred after a week of treatment and the scales disappeared after 14 days of treatment.

In the patients with inverse psoriasis, the lesions were slightly scaly and intensively erythematous with an eroded surface. Cultures were prepared and they were contaminated with Candidas. After 7 days of treatment, scale shedding had ceased and the erythema was reduced. After 14 days of treatment only a slightly erythematous macula was observed.

The most studied case was vulgar psoriasis, because it represented the largest number of patients. The lesions in the trunk area presented considerable infiltration and peripheral scale shedding. Hyperkeratosis was predominant on the articulations. After 7 days of treatment the infiltration and the erythema was drastically reduced. After 14 days reaction was positive on both the trunk and the articulations, and very slightly erythematous lesions were observed on the trunk and slightly scale-shedding lesions on elbows and knees.

In patients with palmo-plantar psoriasis treated with PUVA, the fissures and scale shedding disappeared 72 hours after treatment. In patients with vulgar psoriasis treated with PUVA, the lesions showed no infiltration and scale shedding after 2 sessions.

Example 4. Effects of *Curcuma longa* extracts on the secretion of interleukins IL-6 and IL-8 in human keratinocyte cultures.

Culture of the HaCat line:

The HaCat line is an immortalised line of normal human keratinocytes. These cells grow in a culture medium consisting of Hanks liquid to which 5% of foetal bovine serum and 2% of penicillin-streptomycin is added at 37°C in a CO_2 atmosphere.

Determination of the interleukins.

After 48 hours of incubation with or without radiation, the supernadant of the cultures is taken to measure the IL-6 and IL-8 using an ELISA test kit. The minimum detection for each test is 3.13 pg/ml for the IL-6 and 31.0 pg/ml for the IL-8.

Cell radiation.

The cells were radiated by an UVA/UVB lamp with a UVA range of 340-390 nm and a UVB range of 290-310, with no UVC. The radiation dose was 150 mJ/cm^2. To avoid toxic products from the culture media from forming, PBS free calcium and magnesium ions were changed before radiation.

Results:

The hydroalcoholic and aqueous Curcuma extracts, at doses of 50 µg/ml, inhibited the secretion of interleukins IL-6 and IL-8 after radiation with UVB light in a similar way to betametasone-17-valerate. Figure 1, 2

Example 5. Effect of *Curcuma longa* extracts on the incorporation of BrdU in the DNA of human keratinocytes.

Culture of the HaCat line:

The HaCat line is an immortalised line of normal human keratinocytes. These cells grow in a culture medium consisting of Hanks liquid to which 5% of foetal bovine serum and 2% of penicillin-streptomycin is added at 37°C in a CO_2 atmosphere.

Incorporation of BrdU:

To determine the replication rate, the cells were grown in microplates at a density of $2*10^4$ cells per matrix. After 24 hours of treatment the media was renewed and the cultures were incubated for 24 hours at 37°C with different concentrations of the extracts and betametasone-17-valerate with a concentration of 10 µg/ml. Parallel controls were carried out with the solvent (ethanol 0.1%). The incorporation of BrdU was determined with the ELISA test.

Results:

The incubation of the cells with 50 µg/ml of hydroalcoholic extract leads to a significant decrease in the incorporation with BrdU. The hydroalcoholic extract, a combination of the aqueous and alcoholic extract of *Curcuma longa*, showed an antiproliferative activity similar to betametasone-17-valerate. Figure 3

Example 6 . Effect of *Curcuma longa* extracts on the incorporation of BrdU in the DNA of human keratinocytes after radiation with ultraviolet light.

Culture of the HaCat line

The HaCat line is an immortalised line of normal human keratinocytes. These cells grow in a culture medium consisting of Hanks liquid to which 5% of foetal bovine serum and 2% of penicillin-streptomycin is added at 37°C in a CO_2 atmosphere.

Incorporation of BrdU:

To determine the replication rate, the cells were grown in microplates at a density of $2*10^4$ cells per matrix. After 24 hours of treatment, the media was renewed and the cultures were incubated for 24 hours at 37°C with different concentrations of the extracts and different concentrations of the curcumin and psoralen. Parallel controls were carried out with the solvent (ethanol 0.1%). The incorporation of BrdU was determined with the ELISA test.

The cells were radiated with UVA light at an intensity of $1J/cm^2$ and the incorporation of BrdU was then analysed.

Results:

The hydroalcoholic extract of *Curcuma longa* with 10% curcuminoids, 18% proteins and 3% polysaccharide fraction, showed a photosensitivisation activity greater than curcumin after radiation with UVA light, that is less percentage of incorporation.

Aqueous *Curcuma longa* extract has photosensitivisation properties.

To produce the same level of photosensitivisation as Curcuma extracts, toxic doses of psoralen (10 ng/ml) have to be used. Figures 4, 5,6, 7.

Example 7. Effect of the hydroalcoholic extracts of *Curcuma longa* on psoriasis with visible radiation.

Quantitative composition of the pharmaceutical product:

Hydroalcoholic Curcuma extract *	2 %
Greasy phase	27 %
Emulgents	47 %
Humectants	20 %
Preservatives	1%
pH adjusters	1%
Water	sq

* Equivalent to 10% of curcuminoids, 18% of proteins.

8 patients who were affected and diagnosed with and treated for different types of psoriasis: Guttate, Vulgar, Inverse and Palmo-plantar. The previous treatment consisted of the application of corticoid creams, PUVA sessions (around 14 sessions per patient) and in some cases retinoid therapy. The distribution of the patients by sex, age and type of psoriasis was as follows.

Age	Sex	Type of psoriasis
9	Female	Palmar psoriasis
6	Female	Guttate psoriasis
31	Male	Vulgar psoriasis
46	Female	Vulgar psoriasis
19	Female	Palmo-plantar psoriasis
56	Female	Vulgar and palmo-plantar psoriasis
14	Female	Guttate psoriasis
28	Male	Inverse psoriasis

The cream with the hydroalcoholic extract was applied to the lesions and after 10 minutes the patients were radiated with a 440 nanometer lamp for three minutes. Sessions were weekly. The erythema, the infiltration and the scale shedding was evaluated after 48 hours, 5 days and 15 days.

The results obtained were:

	Day 0		
Type of psoriasis	Erythema	Infiltration	Scale shed
Palmar psoriasis	++	+++	+++
Guttate psoriasis	++	+++	++
Vulgar psoriasis	++	++	+++
Palmo-plantar psoriasis	+	+++	+++
Inverse psoriasis	+++	++	+
	Day 2		
Type of psoriasis	Erythema	Infiltration	Scale shed
Palmar psoriasis	++	++	++
Guttate psoriasis	++	++	+

Vulgar psoriasis	+	++	+++
Palmo-plantar psoriasis	+	+	++
Inverse psoriasis	++	++	+

Day 5

Type of psoriasis	Erythema	Infiltration	Scale shed
Palmar psoriasis	++	+	+
Guttate psoriasis	+	+	-
Vulgar psoriasis	+	+	+
Palmo-plantar psoriasis	+	-	++
Inverse psoriasis	-	+	-

Day 15

Type of psoriasis	Erythema	Infiltration	Scale shed
Palmar psoriasis	+	-	-
Guttate psoriasis	+	-	-
Vulgar psoriasis	-	-	+
Palmo-plantar psoriasis	-	-	+
Inverse psoriasis	-	-	-

+++ intense

++moderate

+slight

- negative

An improvement was observed in the erythema, infiltration and skin shedding after treatment with *Curcuma longa* extract and visible light.

Example 8 . Effect of *Curcuma longa* extracts on the incorporation of BrdU in the DNA of human keratinocytes after radiation with visible light

Culture of the HaCat line

The HaCat line is an immortalised line of normal human keratinocytes. These cells grow in a culture medium consisting of Hanks liquid to which 5% of foetal bovine serum and 2% of penicillin-streptomycin is added at 37°C in a CO_2 atmosphere.

Incorporation of BrdU:

To determine the replication rate, the cells were grown in microplates at a density of $2*10^4$ cells per matrix. After 24 hours of treatment, the media was renewed and the cultures were incubated for 24 hours at 37°C with different concentrations of the extracts and different concentrations of the curcumin and psoralen. Parallel controls were carried out with the solvent (ethanol 0.1%). The incorporation of BrdU was determined with the ELISA test.

The cells were radiated with visible light using an actinium lamp with a spectrum of 400-550 nm (maximum at 450 nm)

Results:

The hydroalcoholic extract of *Curcuma longa* with 10% of curcuminoids, 18% of proteins and 3% of polysaccharide fraction showed a decrease in DNA synthesis. The maximum inhibition of BrdU incorporation was at concentrations of 10 µg/ml of extract. Figure 8.

CLAIMS

1.- Use of a hydroalcoholic extract of *Curcuma longa*, consisting of an alcoholic extract that contains curcuminoids and an aqueous extract that contains a protein fraction, for the manufacture of a pharmaceutical product as a photosensitivisation agent.

2.- Use of an aqueous extract that contains a protein fraction of *Curcuma longa* for the manufacture of a pharmaceutical product as a photosensitivisation agent.

3.- Use of a hydroalcoholic extract of *Curcuma longa*, consisting of an alcoholic extract that contains curcuminoids and an aqueous extract that contains a protein fraction, for the manufacture of a pharmaceutical product as an antiproliferative agent.

4.- Use of an aqueous extract that contains a protein fraction of *Curcuma longa* for the manufacture of a pharmaceutical product as an antiproliferative agent.

5.- Use of a hydroalcoholic extract of *Curcuma longa*, consisting of an alcoholic extract that contains curcuminoids and an aqueous extract that contains a protein fraction, for the manufacture of a pharmaceutical product to inhibit the secretion of cytokines IL-6 and IL-8 after radiation with ultraviolet light.

6.- Use of an aqueous extract of *Curcuma longa* containing a protein fraction, for the manufacture of a pharmaceutical product for the treatment of psoriasis.

7.- Use of a hydroalcoholic extract of *Curcuma longa*, consisting of an alcoholic extract that contains curcuminoids and an aqueous extract that contains a protein fraction, for the manufacture of a pharmaceutical product as a fibrinogen reducer.

8.- Use of an extract of *Curcuma longa*, consisting of an aqueous extract that contains a protein fraction and an alcoholic extract that contains curcuminoids for the manufacture of a pharmaceutical product as a reducer of the Apolipoprotein B/Apolipoprotein A-I quotient.

9.- Use of a hydroalcoholic extract of *Curcuma longa* for the manufacture of a pharmaceutical product, in accordance with claim 1, where the photosensitivisation activity is after radiation with visible light.

10.- Use of an aqueous extract of *Curcuma longa* for the manufacture of a pharmaceutical product, in accordance with claim 2, where the photosensitivisation activity is after radiation with visible light.

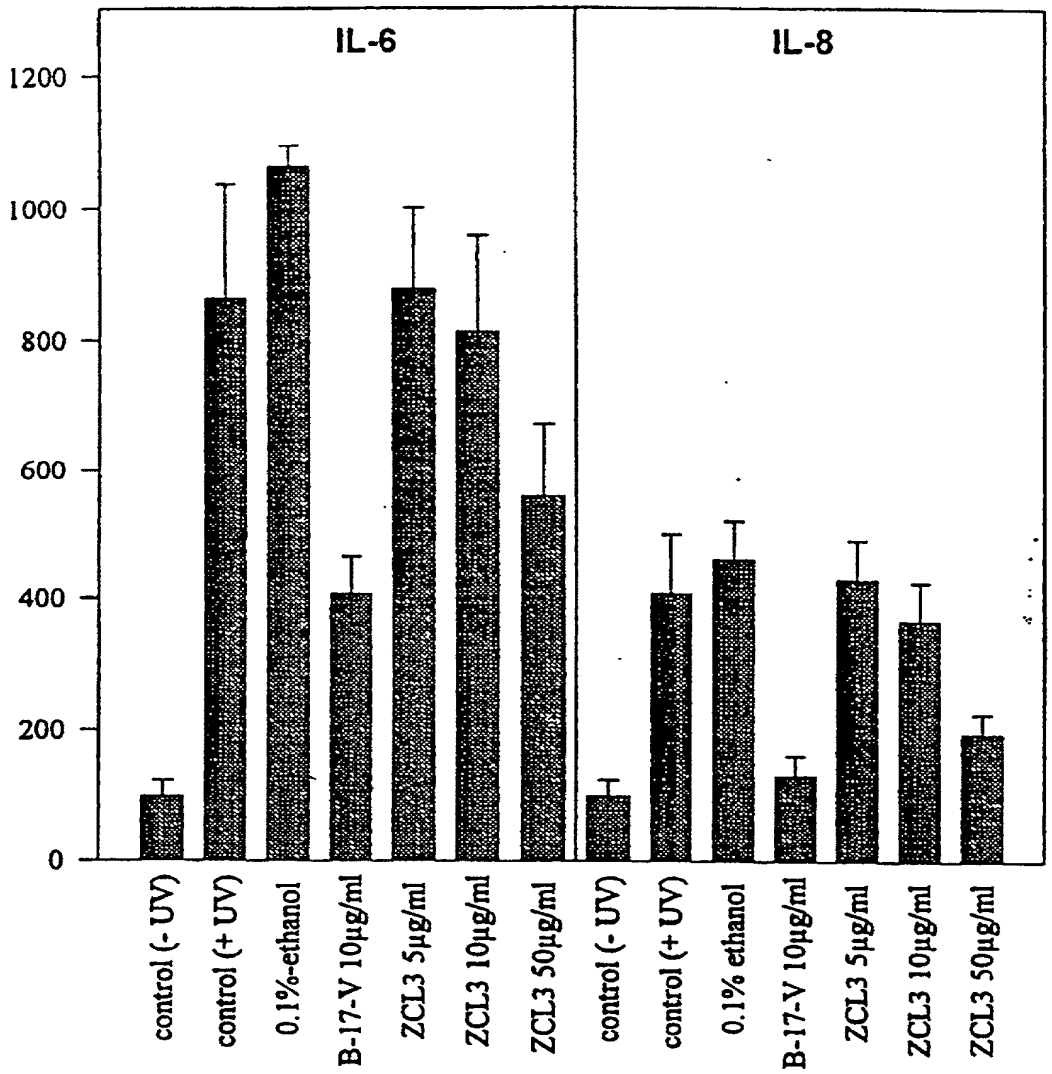

IL-6
IL-8
1200
1000
800
600
400
200
0
control (- UV)
control (+ UV)
0.1%-ethanol
B-17-V 10µg/ml
ZCL3 5µg/ml
ZCL3 10µg/ml
ZCL3 50µg/ml
control (- UV)
control (+ UV)
0.1% ethanol
B-17-V 10µg/ml
ZCL3 5µg/ml
ZCL3 10µg/ml
ZCL3 50µg/ml

FIG. 1

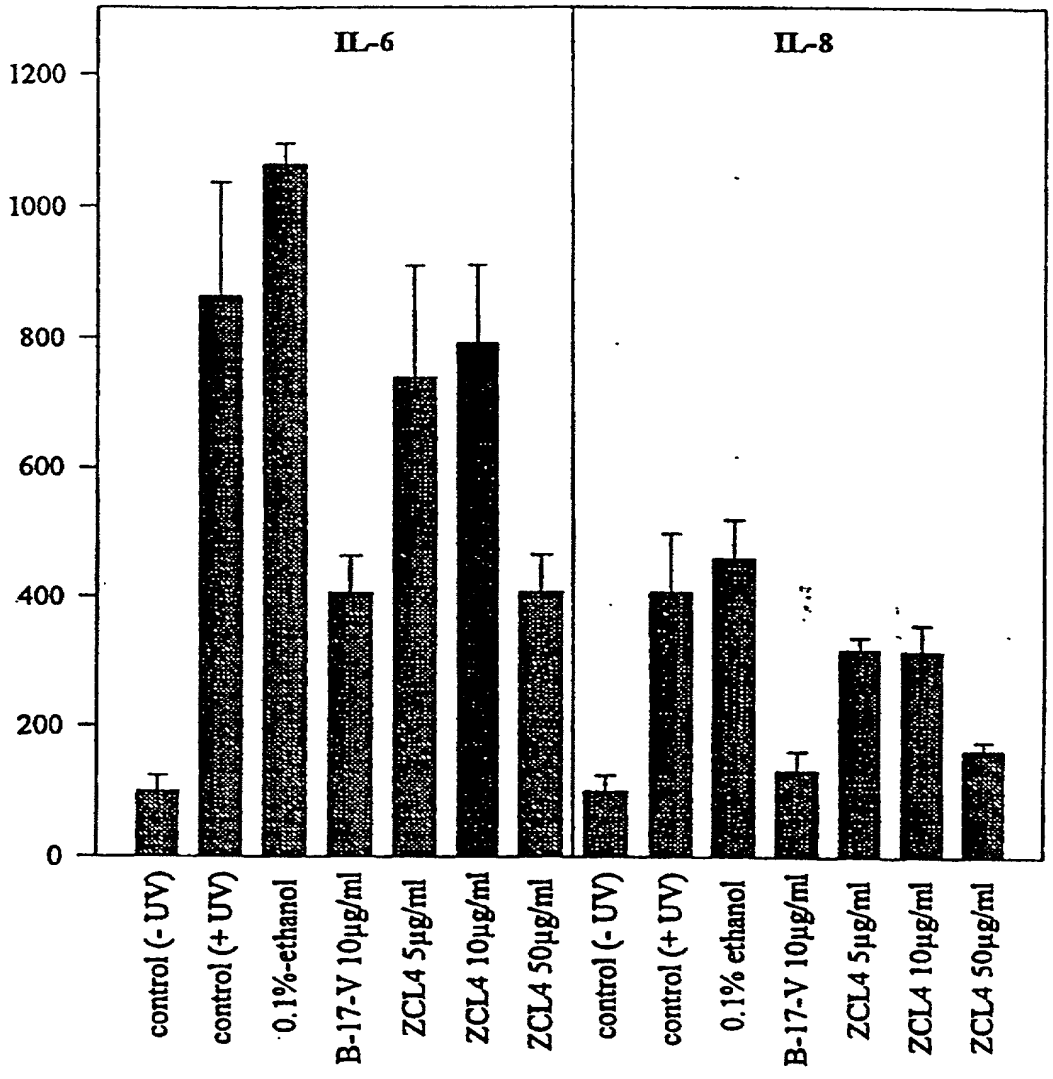


FIG. 2

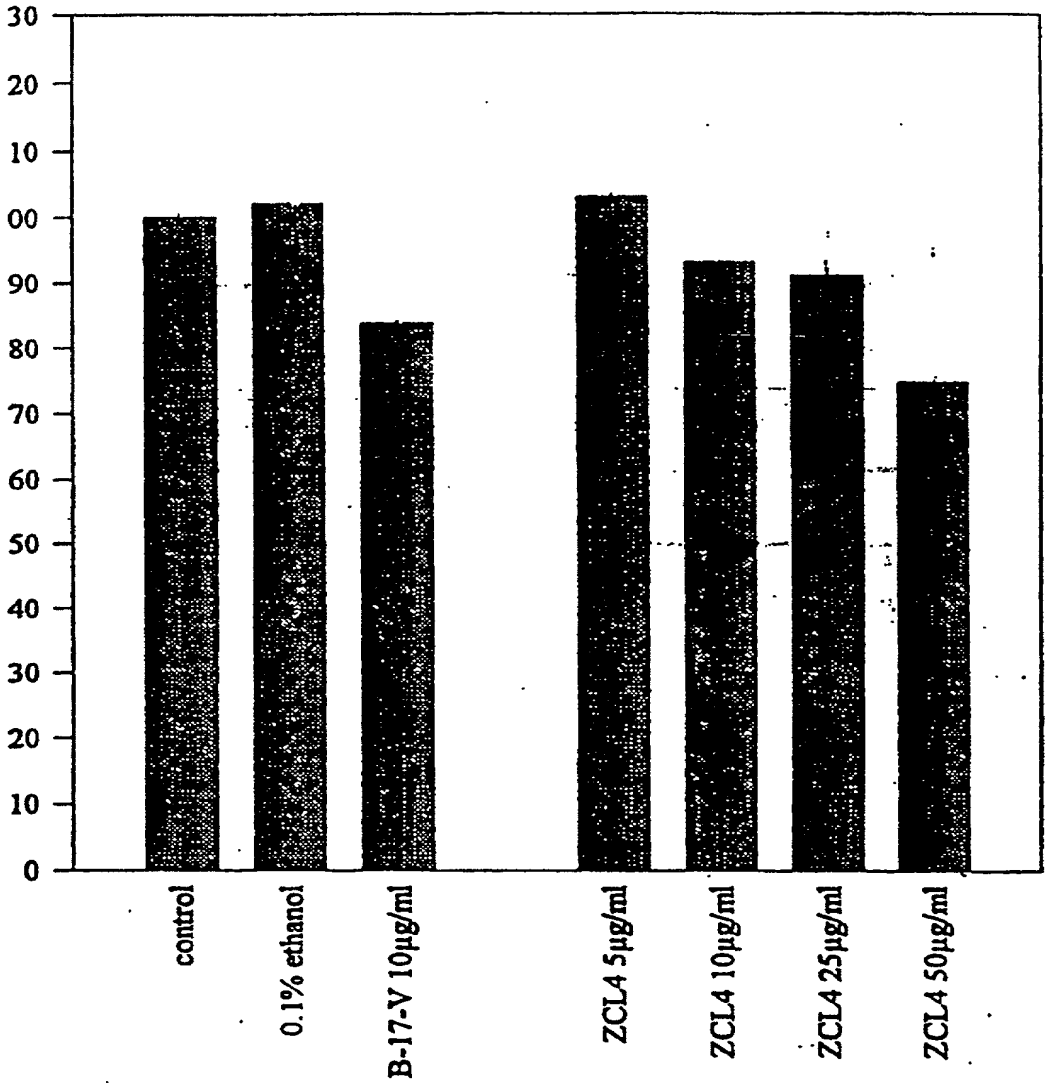

30
20
10
00
90
80
70
60
50
40
30
20
10
0
control
0.1% ethanol
B-17-V 10µg/ml
ZCL4 5µg/ml
ZCL4 10µg/ml
ZCL4 25µg/ml
ZCL4 50µg/ml

FIG. 3

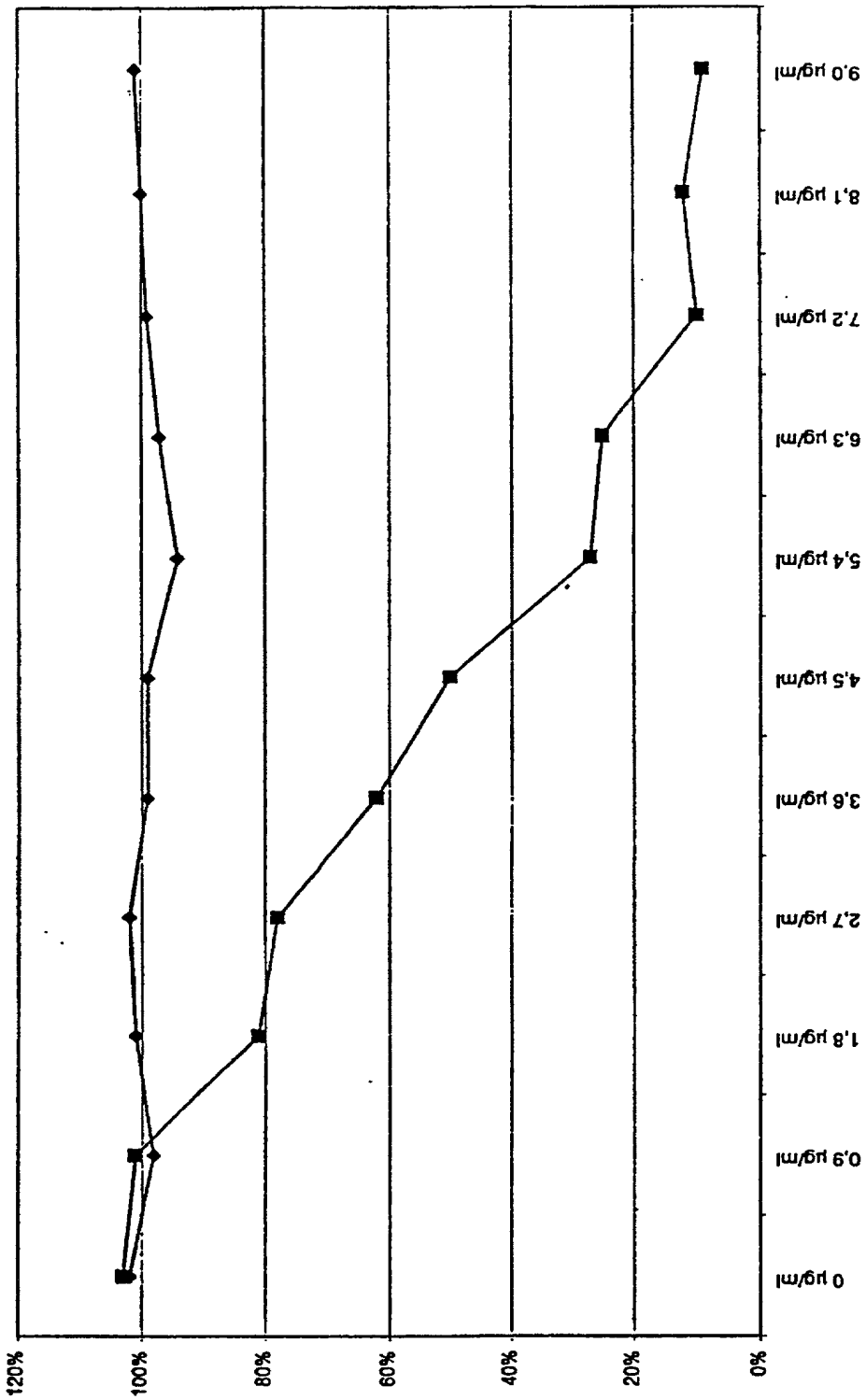

120%
100%
80%
60%
40%
20%
0%
0 µg/ml
0,9 µg/ml
1,8 µg/ml
2,7 µg/ml
3,6 µg/ml
4,5 µg/ml
5,4 µg/ml
6,3 µg/ml
7,2 µg/ml
8,1 µg/ml
9,0 µg/ml

FIG. 4

FIG. 5

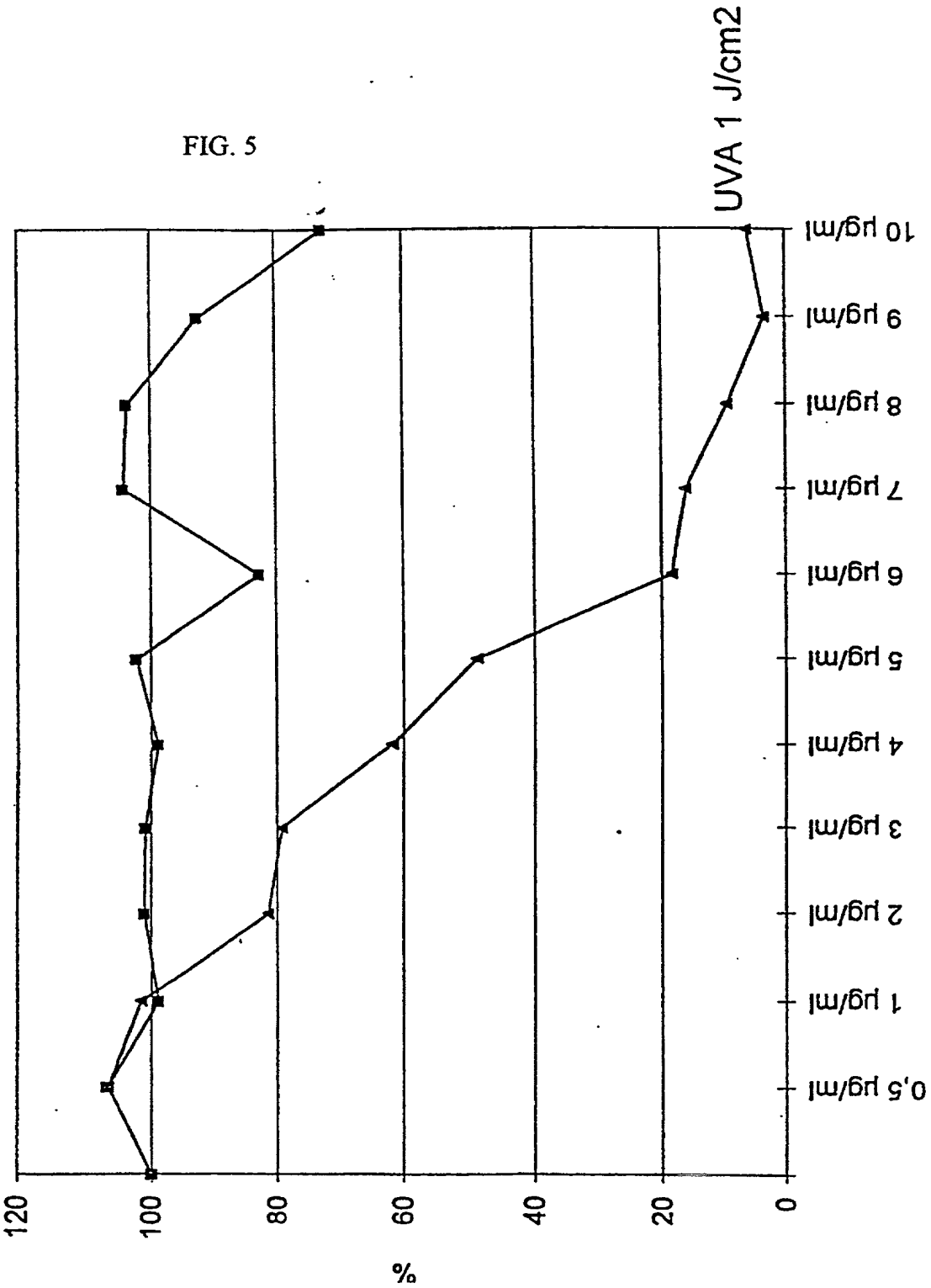


FIG. 6

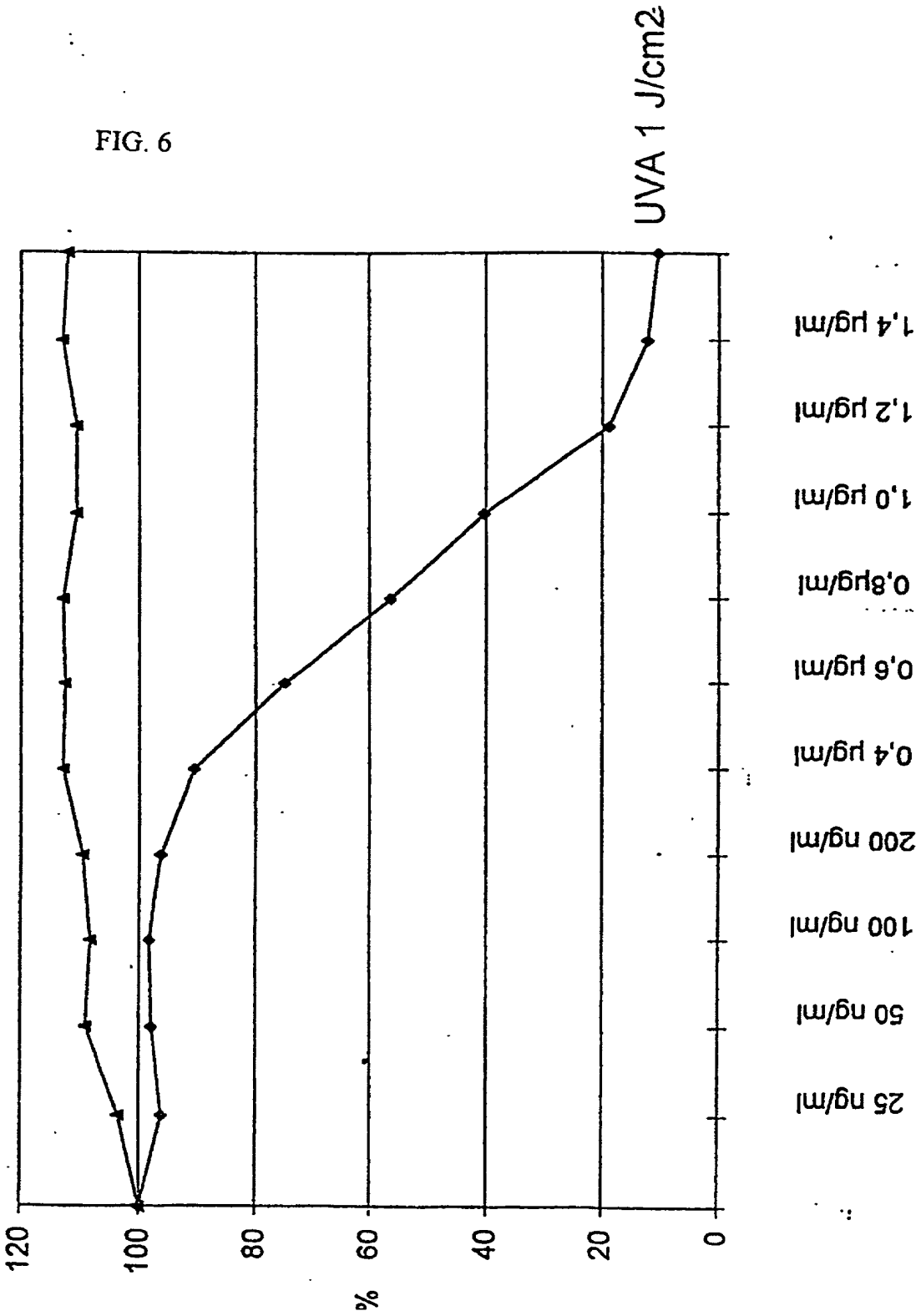
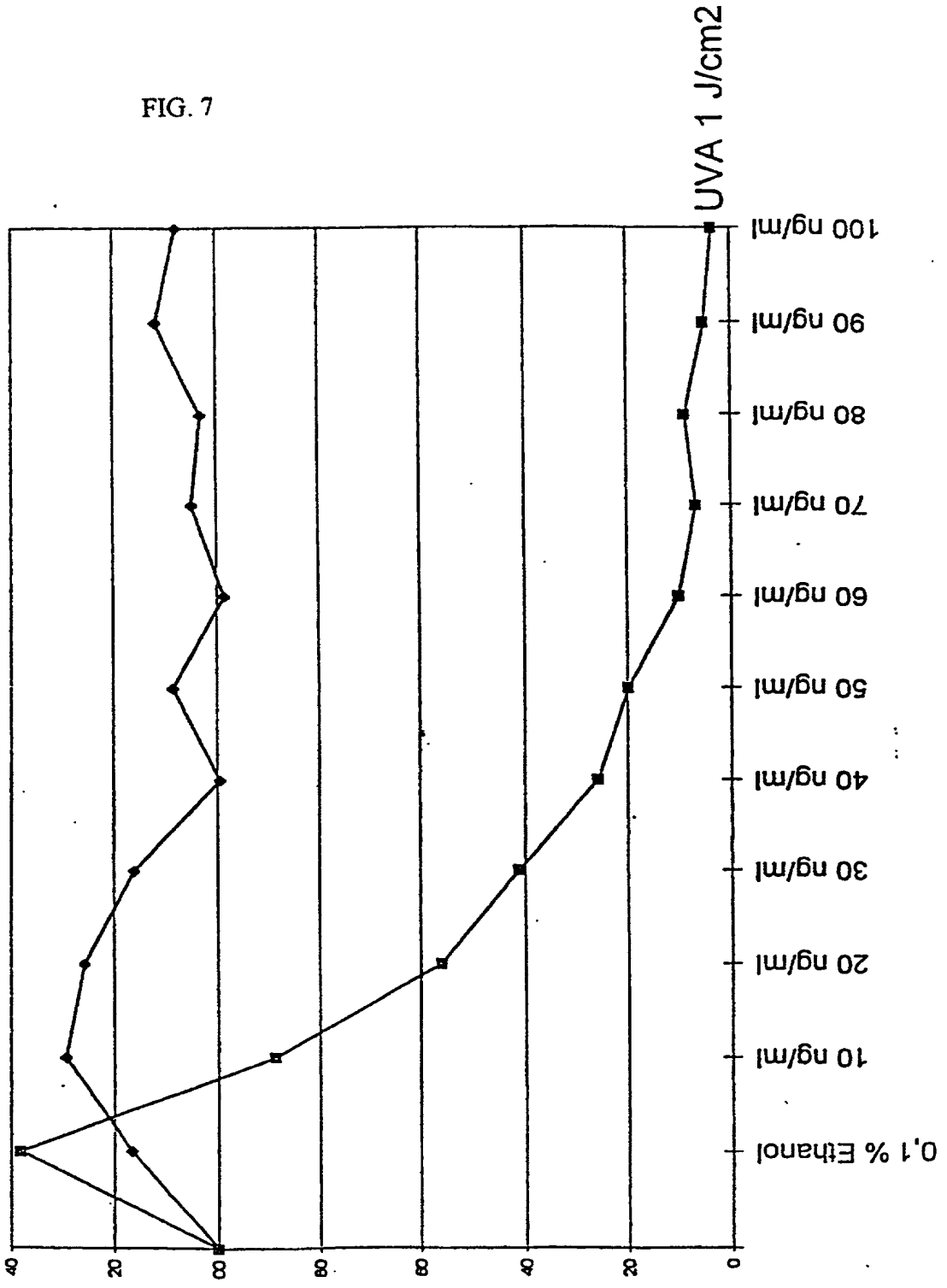


FIG. 7



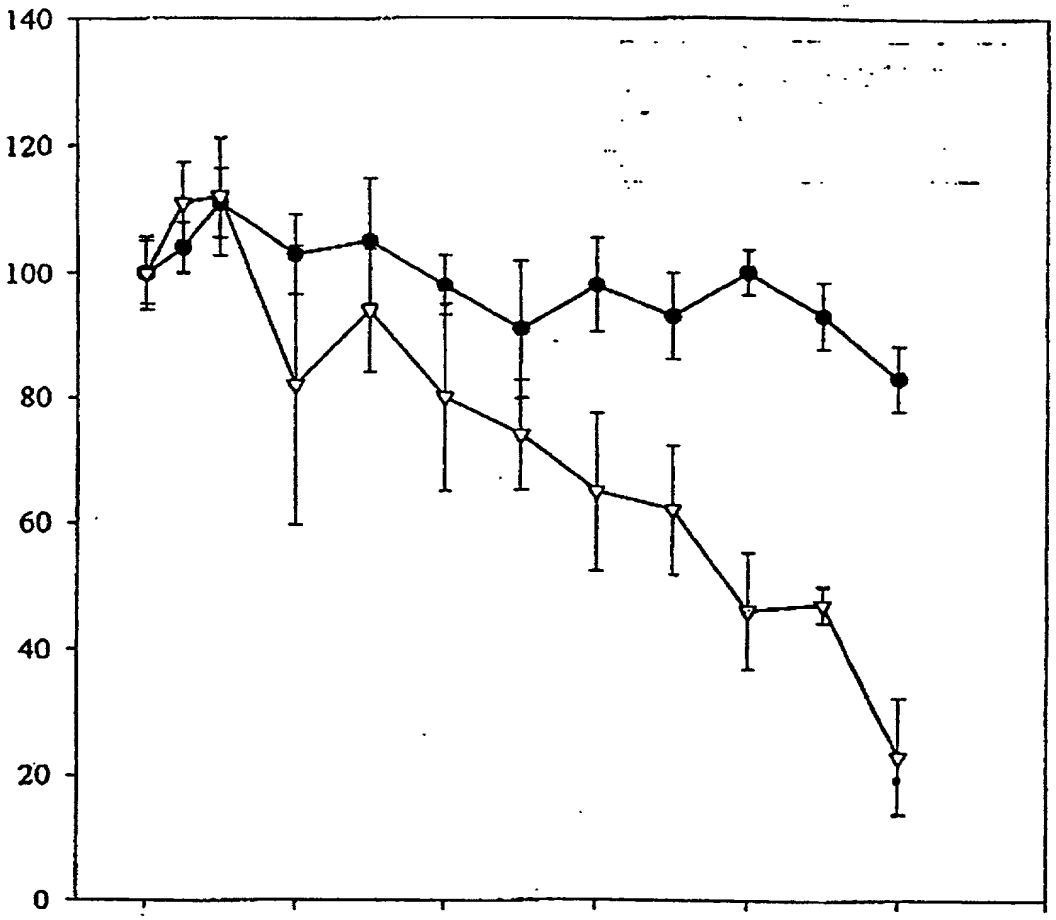


FIG. 8

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PCT

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; that I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

"NEW PHARMACOLOGICAL ACTIVITIES OF CURCUMA LONGA EXTRACTS"

which application is:

☐ the attached application (for original application)

PCT/ES00/00354, filed September 21, 2000

☑ application Serial No. 09/856,035 filed ~~09/856,035~~ E.9.A. on May 17, 2001

(for Declaration not accompanying application)

that I have reviewed and understand the contents of the specification of the above-identified application, including the claims, as amended by any amendment refered to above; that I acknowledge my duty to disclose information of which I am aware which is material to the patentability of this application under 37 C.F.R. 1.56., that I hereby claim priority benefits under Title 35, United States Code §119, §172 or §365 of any provisional application or foreign application(s) for patent or inventor's certificate listed below and have also identified on said list any foreign application for patent or inventor's certificate of this invention having a filing date before that of any foreign application on which priority is claimed:

Application Number	Country	Filing Date	Priority Claimed (yes or no)
P 9902364	Spain	September 23, 1999	YES

I hereby claim the benefit of Title 35, United States Code §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in a listed prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112,, I acknowledge my duty to disclose any information material to the patentability of this application under 37 C.F.R. 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)
PCT/ES00/00354	Sept. 21, 2000	Published

I hereby appoint John H Mion, Reg. No. 18,879; Thomas J. Macpeack, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D.Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Wadell A. Biggart, Reg. 24861; Louis Gubinsky, Reg. No. 24,835; Neil B Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26, 916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg.No. 25, 430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Keneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31, 484; Mark Boland, Reg. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon, Reg. No. 32,778; Abraham J. Rosner, Reg. No. 33, 276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102; Brett S. Sylvester, Reg. No. 32,765, and Robert M. Masters, Reg. No. 35,603 my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office therewith, and request that all correspondence about the application be addressed to SOGHRUE, MION, ZINN, MACPEACK & SEAS PLLC, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037-3202.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Decl. & Pwr 2688 5.1/05

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